P-Element Repression in *Drosophila melanogaster* by a Naturally Occurring Defective Telomeric *P* Copy

Laurent Marin,[1] Monique Lehmann, Danielle Nouaud, Hassan Izaabel,[2] Dominique Anxolabéhère and Stéphane Ronsseray

Département Dynamique du Génome et Evolution, Institut Jacques Monod, 75251 Paris Cedex 05, France

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ABSTRACT

In *Drosophila melanogaster*, hybrid dysgenesis occurs in progeny from crosses between females lacking *P* elements and males carrying *P* elements scattered throughout the genome. We have genetically isolated a naturally occurring *P* insertion at cytological location 1A, from a Tunisian population. The *Nasr'Allah-P*(1A) element [*NA-P*(1A)] has a deletion of the first 871 bp including the *P* promoter. It is flanked at the 3′ end by telomeric associated sequences and at the 5′ end by a *HeT-A* element sequence. The *NA-P*(1A) element strongly represses dysgenic sterility and *P* transposition. However, when testing *P*-promoter repression, *NA-P*(1A) was unable to repress a germinally expressed *P-lacZ* construct bearing no 5′-homology with it. Conversely, a second *P-lacZ* construct, in which the fusion with *lacZ* takes place in exon 3 of *P*, was successfully repressed by *NA-P*(1A). This suggests that *NA-P*(1A) repression involves a homology-dependent component.

THE *P*-transposable element is a recent invader of natural populations of *Drosophila melanogaster*. It is thought to have entered the genome of this species within the last 50 years (Kidwell 1983). Strains that possess *P* elements are called P strains; strains that do not are called M strains. When P males are crossed to M females, the resulting progeny exhibit a syndrome of germline abnormalities (Kidwell *et al.* 1977) due to *P*-element activity. This syndrome, called hybrid dysgenesis, is repressed by various mechanisms (Engels 1989). In some populations, repression is maternally inherited—a condition called P cytotype (Engels 1979). In others, it is biparentally transmitted (Kidwell 1985; Black *et al.* 1987; Simmons *et al.* 1990). Of the 30–60 *P* copies present in a P-strain genome, one-third are complete *P* elements (Bingham *et al.* 1982; O'Hare and Rubin 1983; O'Hare *et al.* 1992). These elements can produce the transposase required for their mobility (Karess and Rubin 1984; Rio *et al.* 1986) and are therefore autonomous. The other two-thirds of the *P* elements in a P-strain genome are nonautonomous because they are structurally incomplete; however, many of these defective elements can be mobilized *in trans* by complete *P* elements (Engels 1984, 1989) because they possess the sequences recognized by the P transposase.

P cytotype represses transcription from the *P* promoter (Lemaitre and Coen 1991; Lemaitre *et al.* 1993; Coen *et al.* 1994). Although in the short term the cytotype is maternally determined, in the long term, it is chromosomally determined by the *P* elements themselves (Engels 1979, 1989). Transformation of an M genome with *P* elements can induce the development of *P* repression over generations (Preston and Engels 1989). Conversely, the removal by segregation of the *P* elements of a P strain leads to the loss of repression capacities in the progeny (Sved 1987). The repression ability of a *P* element depends on its structure and its insertion site (Robertson and Engels 1989; Misra and Rio 1990; Higuet *et al.* 1992; Gloor *et al.* 1993; Misra *et al.* 1993; Rasmusson *et al.* 1993). We have previously sought to identify regulatory *P* elements in the chromosomes of natural populations (Ronsseray *et al.* 1989; Biémont *et al.* 1990) because selection has probably favored their retention. We isolated, in a genomic context devoid of other *P* elements, a pair of autonomous *P* elements inserted near the telomere of an *X* chromosome (cytological site 1A on the polytene chromosomes) from a Russian natural population (Ronsseray *et al.* 1991). The resulting line, called *Lk-P*(1A), can completely repress *P*-induced hybrid dysgenesis, *P* transposition, and transcription from the *P*-element promoter in germline cells (Ronsseray *et al.* 1991; 1996; Lemaitre *et al.* 1993). Furthermore, this repression is maternally transmitted but is lost if the *P* elements at 1A are removed by segregation (Ronsseray *et al.* 1993). The *Lk-P*(1A) *P* elements are inserted in telomeric asso-

All the members of the laboratory render homage to the first author of this article, Laurent Marin, who died in a tragic accident in December 1998.

Corresponding author: Stéphane Ronsseray, Département Dynamique du Génome et Evolution, Institut Jacques Monod, Université Paris 7, 2 place Jussieu, 75251 Paris Cedex 05, France.
E-mail: ronsseray@ijm.jussieu.fr

[1] Deceased.

[2] *Present address:* Université Ibnou-Zohr, Faculté des Sciences. BP28/S, 80000, Agadir, Morocco.

ciated sequences (*TAS*; Karpen and Spradling 1992; Ronsseray *et al.* 1996), which have some properties of heterochromatin, including the ability to silence transgenes inserted within them (Wallrath and Elgin 1995; Cryderman *et al.* 1999). In addition, the repression ability of the *P*(1A) elements is sensitive to the dosage of HP1, a nonhistone heterochromatin protein (James and Elgin 1986; Wustmann *et al.* 1989), which binds mostly to centromeres and telomeres (James and Elgin 1986; James *et al.* 1989; Fanti *et al.* 1998); heterozygosity for a null allele of *Su(var)205*, the gene that encodes this protein, strongly impairs the repression ability of the *Lk-P*(1A) *P* elements (Ronsseray *et al.* 1996, 1997). Genetic and molecular studies show that the *P*(1A) elements are expressed (Ronsseray *et al.* 1991, 1996; Roche *et al.* 1995). This expression is thought to be important for the repression ability of the *Lk-P*(1A) stock because genetic analysis has revealed the existence of a maternally transmitted component of repression termed the "pre-P cytotype" (Ronsseray *et al.* 1993). Indeed, females heterozygous for the *Lk-P*(1A) *P* elements produce oocytes that do not carry the *Lk-P*(1A) elements; however, they transmit in their oocytes a condition that stimulates *P* repression in their progeny.

It is possible that the regulatory properties of the *P*(1A) elements do not depend solely on the expression of these elements. *P-lacZ* insertions at cytological site 1A and a *P-w-ry* insertion at an autosomal telomere (100F) have been shown to prevent the germline expression of a euchromatic *P-lacZ* insertion, a phenomenon termed *trans*-silencing (Roche and Rio 1998), and to stimulate the regulatory properties of other *P* elements in a P-strain genome (Ronsseray *et al.* 1998). These phenomena are strongly associated and have been interpreted to result from chromatin-chromatin interactions involving the telomeric *P* reporter and the euchromatic *P* insertions. However, the telomeric *P*-transgene insertions do not by themselves repress *P*-induced hybrid dysgenesis (Ronsseray *et al.* 1998), probably because of their inability to encode a *P* polypeptide. The regulatory properties of the *P* elements at cytological site 1A are therefore thought to involve both *P*-encoded product(s) and chromatin-chromatin interactions.

In this article, we report the genetic and molecular characterization of a naturally occurring *P* insertion at the 1A site that is derived from a Tunisian population (Nasr'Allah). Unlike previous naturally occurring *P* insertions at 1A, this element [*NA-P*(1A)] has a large deletion at its 5′ end, encompassing both the 31-bp terminal repeat and the *P* promoter. The properties of this new *P*(1A) element are investigated.

MATERIALS AND METHODS

Drosophila stocks: Canton^{y} is a typical M line (Kidwell *et al.* 1977) containing no *P* elements and marked with a spontaneously occurring allele of *yellow*.

Muller-5 is an M line carrying the *Muller-5* (*Basc*) balancer *X* chromosome marked with *Bar* and w^a (Lindsley and Zimm 1992).

Harwich-2 is a P line. The subline used here shows >80 *P* labels by *in situ* hybridization on polytene chromosomes. It has an unidentified autosomal recessive marker (sepia-colored eye), which appeared spontaneously in the stock.

M5/sn^w; π_2 is a P line with the genetic background of the P strain π_2. It carries numerous autonomous *P* elements (Engels 1984) and has both the *Muller-5* balancer chromosome and an *X* chromosome marked with the hypermutable allele sn^w, which causes a slight malformation of the bristles (Engels 1984). This allele is the result of two defective *P* elements inserted at the *singed* locus. In the M cytotype (absence of *P* regulation), sn^w is unstable in the presence of transposase, mutating to sn^e (with a more extreme phenotype) or to sn^+ (wild type). In each case, the change of phenotype is due to the excision of one of the two *P* elements. The excision rate of these *P* elements can be quantified by measuring the instability of sn^w in appropriate crosses.

w cv sn^3 (Umea-30200) is a stock containing an extreme *sn* allele. When made heterozygous with the various derivatives of sn^w, the sn^3 allele enhances their expression and makes scoring easier. The dominance relations of the *singed* alleles are $sn^+ > sn^w > sn^e = sn^3$ (Simmons 1987).

ry^{506} *Sb P*[ry^+ Δ2-3] (99B)/*TM6 Ubx* (abbreviated *Sb* Δ2-3) is a line with the Δ2-3(99B) *P* element linked to a dominant bristle marker (*Stubble*, abbreviated *Sb*).

Birmingham 2/*CyO* (abbreviated *Birm2*/*CyO*) is a strain with numerous defective *P* elements on the *Birm2* second chromosome. It is devoid of repression abilities.

w^{m4} ; *Su(var)2-5*^{04}/*Cy Roi* is a stock with a *Su(var)205* allele that encodes a truncated nonfunctional HP1 protein (Eissenberg *et al.* 1992). This allele strongly impairs the repression ability of *Lk-P*(1A) (Ronsseray *et al.* 1996, 1997).

Nasr'Allah31 ("*N31*") is an inbred line that was collected at Nasr'Allah, Tunisia in 1985 (Izaabel *et al.* 1987). The *N31* line had ~30–40 *P* elements, including a defective copy at the cytological site 1A (Izaabel 1988), and it possessed a maternally inherited *P* regulatory ability that appeared to be correlated with the *P* element at 1A (Izaabel 1988).

P-1152: *P*[$ry^{+t7.2}$ = IArB]A171.1F1; ry^{506} (synonym *WG-1152*) is a line, from Walter Gehring, which carries a *P-lacZ-ry-adh* construct at the cytological site 1A. It is inserted in *TAS* (Roche and Rio 1998). No expression is detected in the female germline, even after an overnight staining.

Gonadal dysgenesis assay: The ability of lines to repress the occurrence of gonadal dysgenic (GD) sterility was measured by the "A* assay" (Kidwell *et al.* 1977). Females of the tested line were crossed with strong P males (Harwich-2). For each test cross, 3–10 pairs were mated *en masse* and immediately placed at 29°. Parents were discarded after 3 days of egg laying. Approximately 2 days after the onset of eclosion, G_1 progeny were collected and allowed to mature for 2 days at room temperature. Twenty-five to 50 G_1 females were then taken at random for dissection. Dissected ovaries were scored as unilaterally dysgenic (S1 type) or bilaterally dysgenic (S0 type; Schaeffer *et al.* 1979). The frequency of gonadal dysgenesis was calculated as %GD = %S0 + ½ %S1 and is referred to as percentage of GD A* (%GD A*). The M cytotype, which allows *P* elements to be active, results in a high percentage of GD A*, whereas the P cytotype, which represses *P*-element activity, results in a low percentage of GD A* (<5%). An intermediate percentage indicates incomplete repression.

***P*-excision assay:** sn^w hypermutability was used to assay the capacity of lines to regulate *P*-element excision in the germline. In the P cytotype, sn^w is almost completely stable even in the presence of transposase. Tested females (10–15) were crossed *en masse* with 15 sn^w; π_2 males at 20°. Fifteen G_1 virgin

females were crossed *en masse* with 15 *w cv* sn^3 males at 25° and allowed to lay eggs in successive bottles for 10 days. Among the G_2 females, sn^e and sn^w individuals were scored. The mutation rate is equal to $u = (sn^e/(sn^e + sn^w)) \times 100$. The absence of regulation results in a high mutation rate, whereas strong regulation results in a low or null mutation rate.

Assay for repression of pupal lethality in the soma: *P*-element transposition is naturally restricted to the germline due to an inhibition of the splicing of the last intron of the transposase gene in the somatic tissues (Laski *et al.* 1986; Siebel and Rio 1990). The Δ2-3 *P* element is an *in vitro*-modified *P* element from which the last intron has been removed. Consequently, this element produces transposase in both the somatic and germline tissues (Robertson *et al.* 1988). Δ2-3 has been inserted at cytological location 99B on chromosome *3*, where it is immobile. In the absence of *P* regulation, Δ2-3 combined with numerous defective *P* elements results in pupal lethality, most likely because of somatic chromosome breakage (Engels *et al.* 1987). In the presence of *P* regulation, this combination does not produce pupal lethality. Twenty females from the line under test were crossed at 18° with 20 *Sb* Δ2-3 males. Twenty G_1 males carrying the Δ2-3 element were crossed with 20 *Birm 2/CyO* females. The G_2 progeny were allowed to develop at 25° and the Sb^+ and Sb phenotypes were scored among the G_2 females that were not Cy. The Sb individuals, which carry Δ2-3, normally die at the pupal stage in the absence of *P* repression, whereas in the presence of *P* repression, they survive. The Sb^+ individuals, which lack Δ2-3, survive in the presence or absence of *P* repression and serve as a viability control. Thus among the non-*Cy* progeny, a percentage of Sb ~50% indicates complete repression of pupal lethality.

Repression of *P-lacZ* expression in ovaries: G_1 females derived from a cross between tested line females and males from a line bearing a transgenic *P-lacZ* element were examined for their capacity to repress the *P* promoter in ovaries by staining or by a quantitative assay for enzyme activity. Two 5′*P-lacZ* constructs were used. The structures of these constructs are diagrammed along with the results (see Figure 4). In the first one, *P*[*lac*, ry^+]A, *lacZ* is fused in frame with the first 587 bp of *P*, which include exon 0 and part of exon 1. The *BQ16* insertion of this construct was isolated in a screen for female sterile mutations. It is located on the second chromosome and expresses the *P-lacZ* transgene in the germline tissues of the ovaries and testes (J. L. Couderc and F. A. Laski, personal communication). However, the genes adjacent to this insertion are still unidentified. In the second construct, *PLH, lacZ* is fused in frame with the first 2410 bp of *P*, which span from exon 0 to part of exon 3 (Kobayashi *et al.* 1993). This construct is driven by the *hsp70* promoter. Without heat shock, no staining is detected in the female germline even after an overnight staining. When heat-shock induced, the *PLH3* insertion of this construct is expressed in the nurse cells and in the mature oocyte. Staining of ovaries and enzyme assays to measure *lacZ* expression was performed as described in Lemaitre *et al.* (1993). The results of the enzyme assays are given in nanomoles per minute per milligram of protein. High activity indicates an absence of *P* repression and low activity indicates strong *P* repression (Lemaitre *et al.* 1993).

Sensitivity of repression to *Su(var)205*: *NA-P*(1A) females were crossed at 20° with heterozygous males, which had a wild-type allele of *Su(var)205* on a balancer chromosome (*Cy*) and a mutant allele (*Su(var)2-5*^{04}) on a Cy^+ chromosome. The regulatory properties of the two kinds of G_1 females, which had paternally inherited a mutant or a wild-type *Su(var)205* allele, were tested by crossing sets of 5–10 females with P-strain (Harwich-2) males at 29°. For each replicate cross, the GD sterility percentage was determined by the dissection of 25–50 daughters.

Pre-P cytotype assay: The ability of the *NA-P*(1A) line to elicit the pre-P cytotype, a maternally transmitted component of *P* repression (Ronsseray *et al.* 1993), was also tested. The mating scheme is presented along with the results.

Sequences around the *P* elements at 1A: *Primers:* For the primers deriving from the *P* sequence (denoted by the letter P), the number indicates the position in the *P* element (O'Hare and Rubin 1983). Orientations are shown in Figure 1.

P1118: 5′-TTGGTTTCCGGTACCTAAATCG-3′
P1282: 5′-GCGGGGTGTCCGAAAAAACG-3′
P1950: 5′-ACGCATTCTTTTAAATTTGTCATAC-3′
P2751: 5′-CCACGGACATGCTAAGGGTTAA-3′
M13 forward: 5′-GTAAAACGACGGCCAGT-3′
T7: 5′-TACGACTCACTATAGGGCG-3′.

Inverse PCR: The DNA adjacent to the 3′ end of the *P* element was amplified by inverse PCR performed on a *Pst*I digest of genomic DNA from *NA-P*(1A), using primers P1950 and P2751 and annealing temperature of 51°.

PCR with an adaptator: The DNA adjacent to the 5′ end of the *P* element was obtained using the rapid amplification of genomic DNA end method developed by Mizobuchi and Frohman (1993), in which the Bluescript SK plasmid was used as an adaptator. *NA-P*(1A) genomic DNA and Bluescript SK plasmid DNA (5 μg each) were digested overnight with *Ssp*I plus *Eco*RI and with *Hin*cII plus *Bam*HI, respectively, to direct the ligation. PCR was performed on the ligation products using the M13 forward and P1282 primers and an annealing temperature of 53°. A nested PCR was then performed using internal primers M13 and P1118 at an annealing temperature of 56°.

RNA blot hybridization: Total RNA was isolated from sets of 360 pairs of ovaries using the RNAzol reagent (Bioprobe Systems, Montreuil Sous Bois, France). Poly(A)$^+$ RNA was purified by chromatography through an oligo(dT) column, separated by electrophoresis in a 1.3% agarose/formaldehyde gel, and transferred onto a nitrocellulose membrane under conditions recommended by the supplier (Schleicher and Schuell, Keene, NH).

Statistical analysis: The repression capacities as measured by GD sterility percentages were compared using the nonparametric Mann-Whitney test performed on A* assay replicates.

RESULTS

Synthesis of a line with a single *P*-hybridization site at 1A: A line carrying the tip of the *X* chromosome from the inbred *N31* line (Izaabel 1988) in an M-type chromosomal background was synthesized as described previously for the synthesis of the *Lk-P*(1A) line (Ronsseray *et al.* 1991), except that an M line with autosomal balancers was used to substitute the autosomal complement. The resulting *Nasr'Allah-P*(1A) line [*NA-P*(1A)] was labeled only at 1A by *in situ* hybridization with a *P*-element probe (data not shown). This line is genetically marked with a w^{sp} allele.

Molecular analysis of the structure of the *P* element of *NA-P*(1A) and characterization of the adjacent sequences: Figure 1 shows the structure of the canonical *P* element, the probes and primers used in the analysis, and the map of the *NA-P*(1A) *P* element derived from Southern blot analysis and DNA sequencing. Southern blotting has shown that the *NA-P*(1A) *P* element is deleted at its 5′ end. The deletion breakpoint maps be-

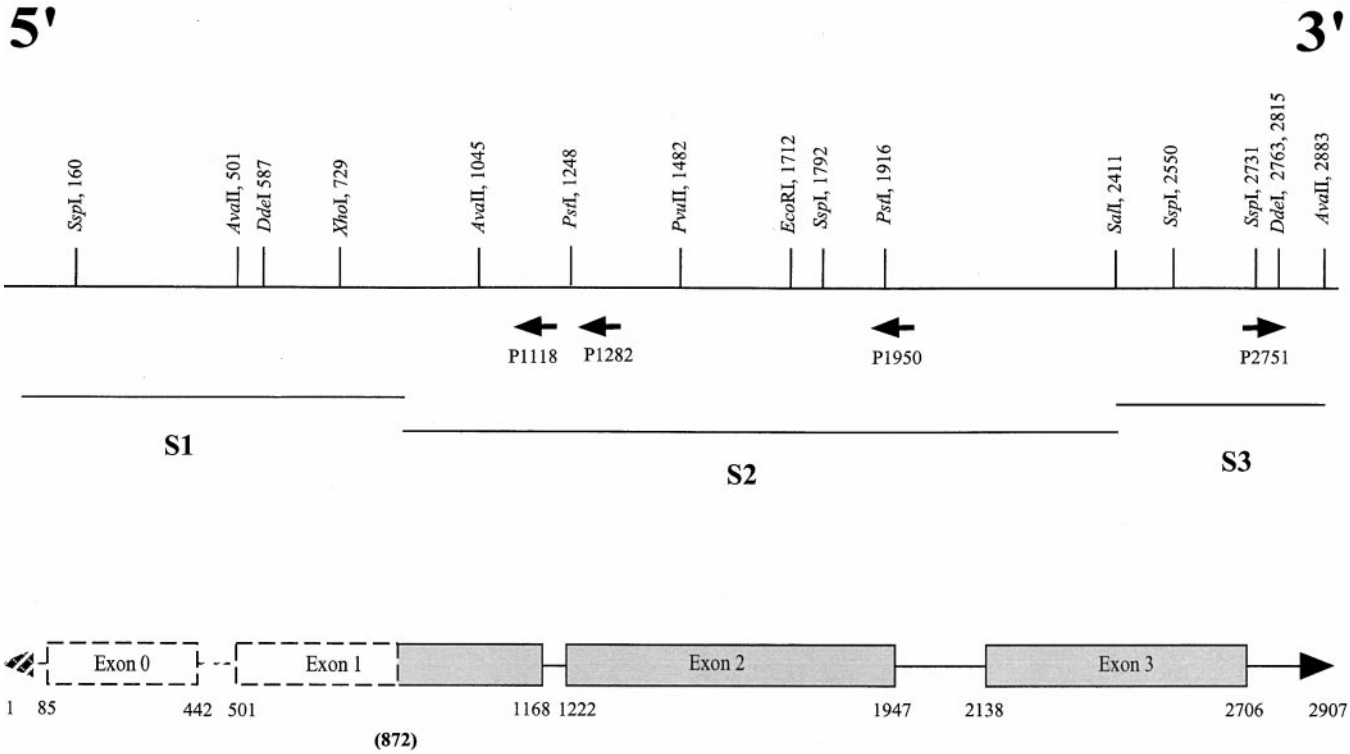

Figure 1.—Structure of the *P* element of *NA-P*(1A). (Top) The restriction map is shown with the probes (S1, S2, S3) used for Southern blot analysis and the primers (solid arrows) used for PCR. All sites from *Ssp*I (160) to *Xho*I (729) were found to be absent in the *NA-P*(1A) element but the sites from *Ava*II (1045) to *Ava*II (2883) were found to be present. (Bottom) The deleted part of *NA-P*(1A) is shown (dashed box) on a canonical *P*-element map (Rio *et al.* 1986). Positions of exons are given below the map with the position of the first nucleotide present in the *NA-P*(1A) element in parentheses. The arrowheads indicate the 31-bp inverted terminal repeats of the *P* element.

tween the *Xho*I site (coordinate 729) and the *Ava*II site (1045). To determine the precise structure of its 5′ and 3′ ends, the *NA-P*(1A) *P* element was partially sequenced using PCR products (Figure 2). The 3′ end was found to be similar to that of the canonical *P* element. At the 5′ end, the first 871 bp of the canonical *P* sequence (O'Hare and Rubin 1983) are missing. The *NA-P*(1A) *P* element therefore lacks the 31-bp 5′ terminal inverted repeat, the *P* promoter (53–103; Kaufmann *et al.* 1989), and the transposase 5′ binding site (48–68; Kaufmann *et al.* 1989). Consequently, it cannot be mobilized by the transposase (Mullins *et al.* 1989; Beall and Rio 1997). Furthermore, this element cannot be transcribed unless transcription is initiated at an external promoter.

Inverse PCR was carried out to analyze the sequences adjacent to the *NA-P*(1A) *P* element (Figure 2). At the 5′ end, the *P* element is flanked by a sequence homologous to the *HeT-A* transposable element (Biessmann *et al.* 1990, 1992a,b; Valgeirsdottir *et al.* 1990). This LINE-like element is specific to Drosophila telomeres (Biessmann *et al.* 1990, 1992a,b; Levis *et al.* 1993) and to the *Y* chromosome (Danilevskaya *et al.* 1992). *HeT-A* elements are known to transpose to the termini of the chromosomes (Biessmann *et al.* 1990, 1992a,b). They are distal to the *TAS* elements and constitute the terminal sequences of the chromosomes. At the 3′ end of the *NA-P*(1A), the flanking sequence was found to be homologous to *X*-linked *TAS* elements (Karpen and Spradling 1992). The adjacent sequence corresponds to a 173-bp internal subrepeat of the *TAS* elements. The maximum level of identity (100%) between the *NA-P*(1A) 3′ adjacent sequence and the *TAS* of Karpen and Spradling (1992) is found in the second internal 173-bp subrepeat of the *TAS* element.

Expression of the *NA-P*(1A) *P* copy: Northern blot

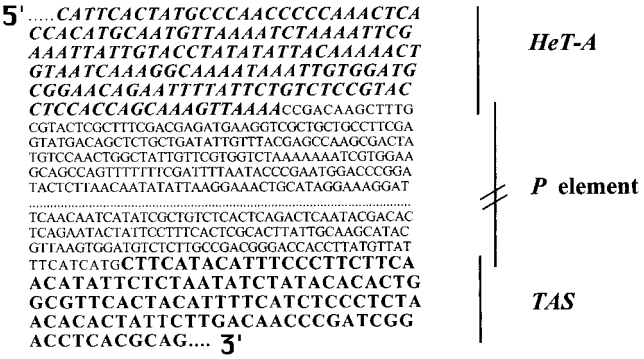

Figure 2.—Sequences flanking the *NA-P*(1A) *P* element. Boldface italic, *HeT-A* homologous sequence; small caps, *P*-homologous sequence; boldface, *TAS*-homologous sequence. The first nucleotide at the 5′ end of the *P* element corresponds to nucleotide 872 in the canonical *P*-element sequence (O'Hare and Rubin 1983).

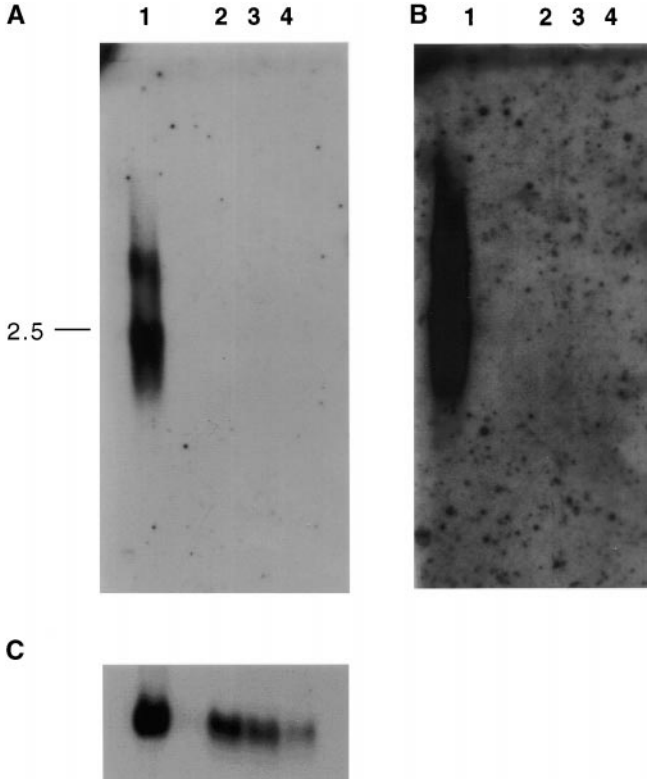

Figure 3.—Northern blot analysis of poly(A)$^+$ RNA in ovaries: Autoradiography was carried out for 26 hr (A) and for 7 days (B). The 2.5-kb *P* transcript is indicated. To check for relative quantities of RNA (C), the same filter was rehybridized with a riboprobe from RP49, a ribosomal protein cDNA (Vaslet *et al.* 1980). Lanes: 1, Harwich-2; 2, *Lk-P*(1A); 3, *NA-P*(1A); 4, Canton^y (M).

TABLE 1

Repression of *P*-element activity

Tested lines	GD A*	*sn*^{w} excision	Pupal lethality	*P-lacZ* activity
Canton^y	100	4.0	0.0	5.6
	0.0	(1159)	(588)	0.5
	(10)			(4)
Harwich-2	0.3	0.0	50.3	—
	0.6	(332)	(1320)	—
	(5)			—
Lk-P(1A)	1.1	0.0	1.2	2.8
	2.0	(820)	(728)	2.5
	(35)			(4)
NA-P(1A)	1.9	0.1	0.4	5.7
	2.9	(2450)	(732)	1.1
	(10)			(4)

GD A*, the mean percentage of GD sterility (first line), the standard deviation over replicates (second line), and the number of replicates performed (in parentheses, third line). For each replicate cross, 50–100 ovaries were examined in the progeny. GD A* results obtained for Harwich-2 and *Lk-P*(1A) are from Ronsseray *et al.* (1998). *sn*^{w} excision, repression of *P* excision at the *singed* locus. The percentage of *sn*^{e} females among *sn*^{e} and *sn*^{w} G_2 females (first line) and the total number of flies scored (in parentheses, second line) are given. Pupal lethality, the percentage of adults carrying the Δ2-3 element (first line) and the total number of adults (in parentheses, second line). *P-lacZ* activity, results are given in nanomoles per milligram of protein. The mean of replicates is given (first line), with the standard deviation (second line), and the number of replicates (in parentheses, third line).

analysis was performed on poly(A)$^+$ RNA extracted from the ovaries of the *Lk-P*(1A) and *NA-P*(1A) lines, the P strain Harwich-2, and the M strain Canton^y. A riboprobe from pS2-KS, which contains the *Hin*dIII-*Sal*I fragment of the *P* element in the genomic DNA clone pπ25.1 (O'Hare and Rubin 1983) inserted in the Bluescript SK+ vector, was hybridized to the blot. Figure 3A shows that, as expected, the M strain Canton^y produced no detectable *P* transcripts. Harwich-2, however, produced two different transcripts, a 2.5-kb transcript corresponding to the product of complete *P* elements and another transcript that was longer (Karess and Rubin 1984). With both *Lk-P*(1A) and *NA-P*(1A), no hybridizing signal was detected after 7 days of exposure (Figure 3B), or even after 26 days (data not shown). However, genetic analysis has shown that the *P* elements of *Lk-P*(1A) are expressed in the germline since this line exhibits a weak but significant transposase activity (Ronsseray *et al.* 1996). Therefore, we cannot exclude that the *NA-P*(1A) element is also expressed at a low level.

Regulatory properties of the *NA-P*(1A) line: The ability of the *NA-P*(1A) line to repress *P*-element activity was tested with four different assays.

Repression of GD A sterility (Table 1, column 1):* G_1 females reared at 29° from the cross of M females (Canton^y) with P males (Harwich-2) are sterile due to atrophy of the gonads (100% gonadal dysgenesis). Conversely, P females [Harwich-2 or *Lk-P*(1A)] crossed with P males produce G_1 females with trivial percentages of GD sterility (0.3 and 1.1%, respectively) due to a repressive component transmitted by the P females. With *NA-P*(1A) females, there is also nearly complete repression of GD sterility (1.9%). The level of repression of this line is as strong as that of *Lk-P*(1A).

Repression of excision of the defective P elements in the unstable singed-weak allele (Table 1, column 2): Both the *sn*^{w} allele and autonomous *P* elements, which supply transposase, were paternally introduced in the G_0. Daughters of M females produce 4% of *sn*^{e} in their progeny whereas daughters of P females [Harwich-2 or *Lk-P*(1A)] produce no *sn*^{e} in their progeny. The daughters of *NA-P*(1A) females produce only 0.1% *sn*^{e} in their progeny. Thus, the *NA-P*(1A) strain is a strong repressor of *P*-element excision.

Repression of P activity in the soma (Table 1, column 3): The combination of the somatically expressed transposase source Δ2-3 and defective *P* elements that lack *P* repression ability causes pupal lethality, probably because of somatic chromosome breakage (Robertson *et al.* 1988). In the absence of *P* regulation, the Sb individuals, which carry Δ2-3, are therefore expected to die whereas in the presence of *P* regulation they are ex-

pected to live. There are no surviving Sb adult flies in the progeny from the M strain Canton^{y} (Table 1, column 3), whereas half of the adult progeny are Sb (50.3%) in the progeny of the P strain Harwich-2. *Lk-P*(1A) and *NA-P*(1A) fail to rescue pupal lethality since 1.2 and 0.4% of Sb flies are produced, respectively. These data are consistent with previous results showing that *P*(1A) lines are almost devoid of somatic repression capacities (Ronsseray *et al.* 1996).

Repression of a P-lacZ element in the germline (Figures 4–6; Table 1, column 4): The *BQ16 P-lacZ* insertion was used initially in this assay. The structure of this *P* reporter is shown in Figure 4; 587 bp of *P* are present upstream of the lacZ fusion. In M strains, this insertion is strongly expressed in the nurse cells and in the mature oocyte (Figure 5A). By contrast, G_1 females from the cross of *Lk-P*(1A) females with *BQ16* males strongly repress *lacZ* activity in the germline (Figure 5B). *P-1152* is an insertion of a *P-lacZ* construct at 1A in *TAS* (see materials and methods) with the first 587 bp of *P* present upstream of the lacZ fusion. Females carrying the *P-1152* transgene also strongly repress *BQ16* expression (Figure 5C), thereby demonstrating that the *trans*-silencing effect previously described with a *P-vasa-lacZ* target (Roche and Rio 1998) also works on a *P-lacZ* target. Surprisingly, G_1 females from the cross between *NA-P*(1A) females and *BQ16* males show strong *lacZ* staining (Figure 5D). Assays of *lacZ* activity were also performed on the ovaries of G_1 progeny from test crosses (tested line females $\times$ *BQ16* males). According to these assays, *NA-P*(1A) fails to repress the *lacZ* activity of *BQ16* (Table 1, column 4).

The failure of *NA-P*(1A) to repress *P-lacZ* expression was also tested by staining assays with two other euchromatic insertions of the same *P-lacZ* construct as in *BQ16*, called *ABOO* and *BA37* (J. L. Couderc and F. Laski, personal communication). Both are located on the second chromosome, at different genomic locations from *BQ16*. *ABOO* is expressed in nurse cells and mature oocytes. Here again, *Lk-P*(1A) repressed the *ABOO P-lacZ* insertion in the germline, whereas *NA-P*(1A) did not (data not shown). This shows that the inability of *NA-P*(1A) to repress is not restricted to the *BQ16* genomic insertion site. *BA37* is expressed in the follicle cells. It is repressed by Harwich but not by *Lk-P*(1A), *P-1152*, or *NA-P*(1A) (data not shown). This result is consistent with the fact that the repression ability of *P*(1A) lines is restricted to the germline (Ronsseray *et al.* 1996).

Because of these results, the repression capacities of *NA-P*(1A) were tested with another *P-lacZ* structure in which the *lacZ* fusion is in exon 3 of the *P* element (*PLH3*, see Figure 4). By contrast to *BQ16*, which shares no 5′ homology with *NA-P*(1A), the *PLH3* construct has >1.5 kb of homology with *NA-P*(1A) upstream of the *lacZ* fusion. Figure 6, A–C, shows that, after a heat shock, the *PLH3* transgene is strongly expressed in the germline and is repressed by both *Lk-P*(1A) and *P-1152*. Furthermore, it is also strongly repressed by *NA-P*(1A) (Figure 6D). This indicates that although *NA-P*(1A) is unable to repress the *P* promoter of the *P-lacZ* construct in *BQ16*, it can repress the *P-lacZ* construct in *PLH3*, suggesting that the homology between the 5′ sequence of the telomeric *P* element and the *P-lacZ* transgene is important.

From the above tests, it appears that *NA-P*(1A) parallels the *Lk-P*(1A) line except for the ability to repress *P-lacZ* expression in the germline of different transgenic strains. The repression capacities as inferred from the different tests are therefore not strictly correlated.

Maternal inheritance assay of repression of GD sterility: Depending on the strains, the regulatory properties in the P-M system can be maternally inherited ("P cytotype"; Engels 1979) or biparentally transmitted ("*P* susceptibility"; Kidwell 1985). The repression ability of *Lk-P*(1A) has been shown to be maternally inherited (Ronsseray *et al.* 1991). Consequently, we tested the inheritance of the *NA-P*(1A) repression ability. M females (Canton^{y}) were crossed to *NA-P*(1A) males (cross termed "M-MI" for M maternal inheritance) and reciprocally, *NA-P*(1A) females were crossed to Canton^{y} males (cross termed "P-MI" for P maternal inheritance) at 20°. In both cases, G_1 females were tested for their regulatory properties by the A* assay and the percentage of GD sterility was determined for G_2 females (Table 2, columns 1 and 2). Data from a similar experiment performed with *Lk-P*(1A) individuals instead of *NA-P*(1A) are also presented. With *Lk-P*(1A), P-MI G_1 females show strong repression ability because they have inherited the P cytotype from their *Lk-P*(1A) mothers; conversely, M-MI G_1 females show almost no repression ability because of the M cytotype inherited from their Canton^{y} mothers. With *NA-P*(1A), the two types of females also differed in their repression abilities (Table 2, columns 1 and 2; $P < 0.01$ by the Mann-Whitney test) but the level of repression found with the P-MI G_1 females from *NA-P*(1A) was weaker than that found with the corresponding females from *Lk-P*(1A) (74.4 *vs.* 2.2%). Nonetheless, these experimental results indicate that *NA-P*(1A) has a maternally transmitted repression ability similar to the P cytotype but much weaker than that of *Lk-P*(1A).

Sensitivity of *NA-P*(1A) repression ability to a mutation in *Su(var)205*: The repression ability of *Lk-P*(1A) is strongly sensitive to mutations in *Su(var)205*, a gene that encodes HP1 (Ronsseray *et al.* 1996). The presence of a null allele of *Su(var)205* abolishes the ability of the *Lk-P*(1A) *P* elements to repress GD sterility. We tested the sensitivity of the repression capacities of *NA-P*(1A) to *Su(var)205* by comparing females carrying two wild-type alleles of *Su(var)205* with their sisters carrying a wild-type and a mutant allele (Table 2, columns 3 and 4). We detected a strong effect. Females that inherit the *NA-P*(1A) *P* element maternally and that carry two wild-type alleles of *Su(var)205* repress GD sterility at a

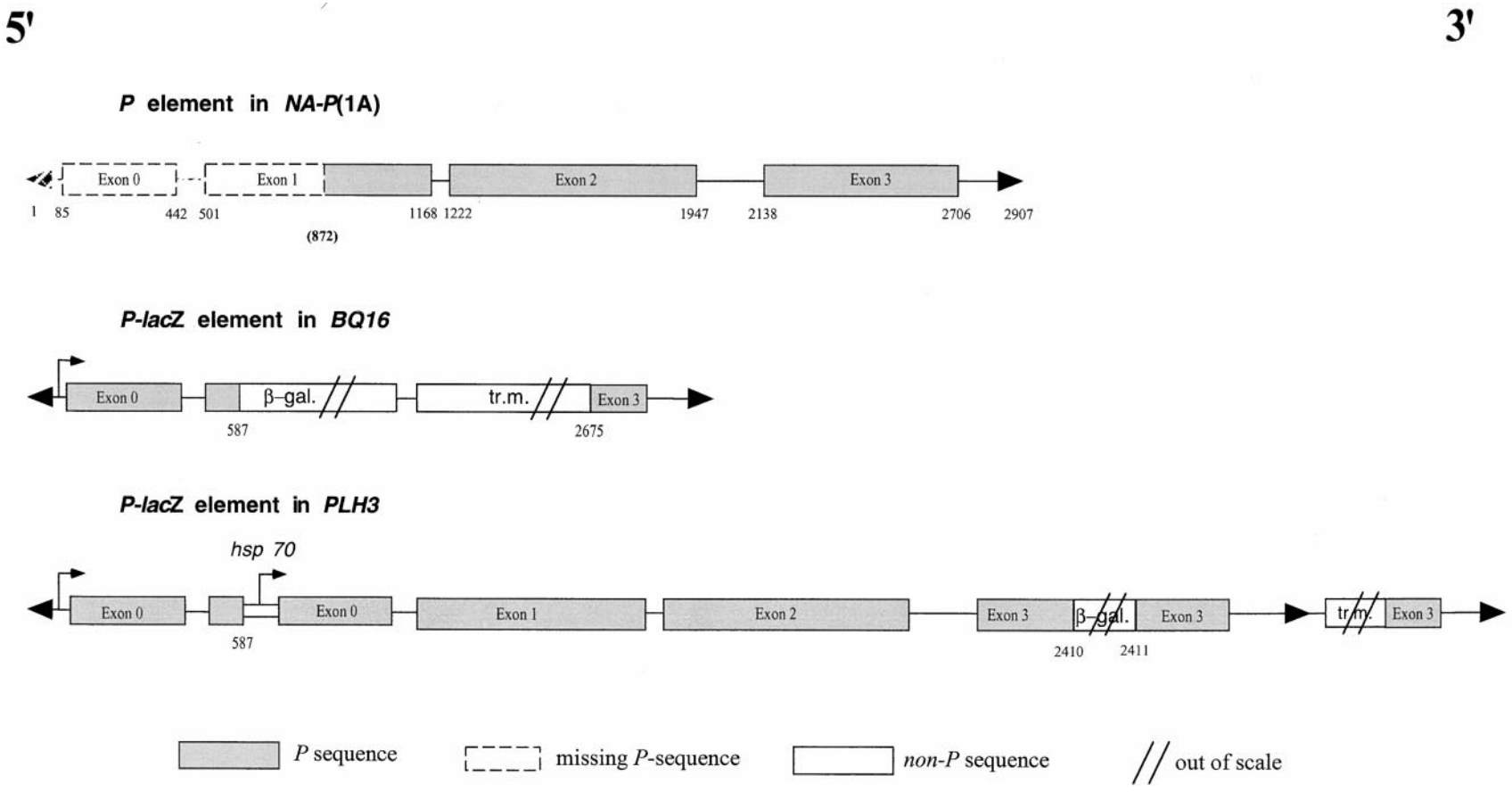

Figure 4.—*P-lacZ* structures used in repression assays. The *NA-P*(1A) element is also shown to allow easier comparisons with the *P-lacZ* structures. *BQ16* and *PLH3* are *P*[*lac*, *ry*$^{+}$]A and *PLH* constructs, respectively. Numbers below the constructs correspond to nucleotide positions in the 2097-bp *P*-element sequence. tr.m., transformation markers.The fragment with the *hsp70* promoter in the construct in *PLH3* is 344 bp long. For a detailed structure of *PLH3*, see Kobayashi *et al.* (1993).

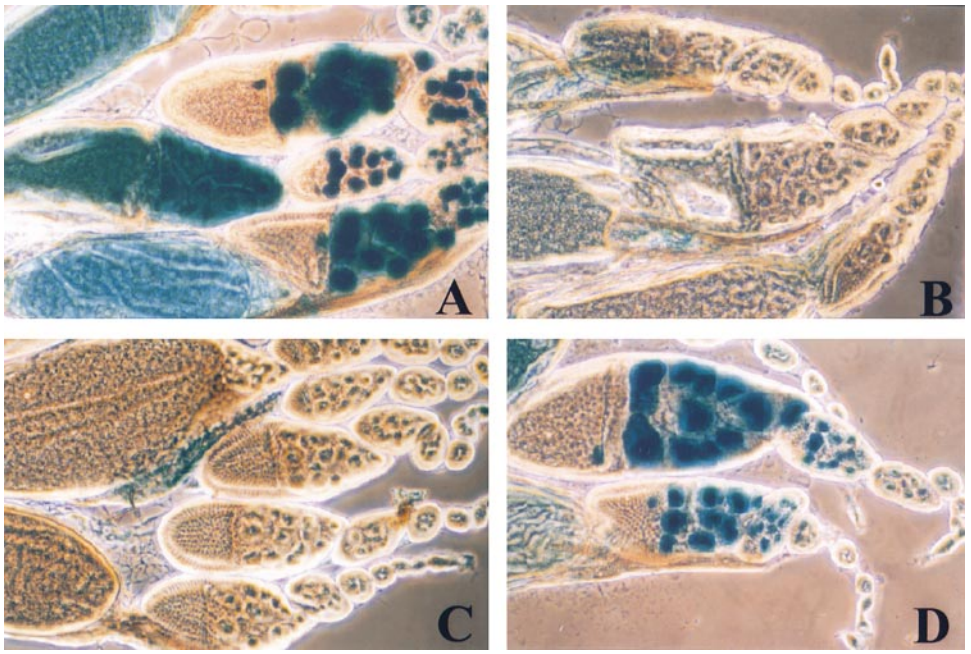

Figure 5.—Repression assay of the *P-lacZ* in *BQ16*. Each picture shows the staining of ovaries of G_1 females from the cross of tested females with *BQ16* males, reared at 20°. Tested females: (A) Canton^y; (B) *Lk-P*(1A); (C) *P-1152*; (D) *NA-P*(1A). Staining reactions were performed overnight in the same experiment to allow a visual comparison. The *BQ16* insertion expresses β-galactosidase only in the ovarian germline tissues (nurse cells and mature oocyte).

level (70.0%) close to that of the P-MI group in the maternal inheritance assay (74.4%, column 1). However, their sisters, which differ only by the presence of a mutated allele of *Su(var)205*, showed little or no repression capacity (95.2% sterility). This difference is highly significant ($P < 0.01$) by the Mann-Whitney test. *NA-P*(1A) repression ability is therefore sensitive to *Su(var)205*.

Thermosensitivity of *NA-P*(1A) repression ability: The determination of *P*-repression capacities in G_1 females from crosses between P and M individuals is strongly thermosensitive (Ronsseray *et al.* 1984; Ronsseray 1986). During imaginal life, an 18° treatment results in a decrease of repression capacities whereas a treatment at temperatures >25° results in an enhancement of repression capacities. These modifications can be at least partially reversible. *Lk-P*(1A) repression is also thermosensitive (Ronsseray *et al.* 1991; data reproduced here in Table 2, columns 5–7). The thermosensitivity of repression ability in G_1 females resulting from crosses between *NA-P*(1A) females and Canton^y males was investigated by aging sets of virgin G_1 females at different temperatures. Table 2 (columns 5–7) shows that the G_1 females, which have partial repression ability (58.3% sterility) at emergence, evolve toward a lack of repression capacities (81.0%) after 15 days at 18° and toward

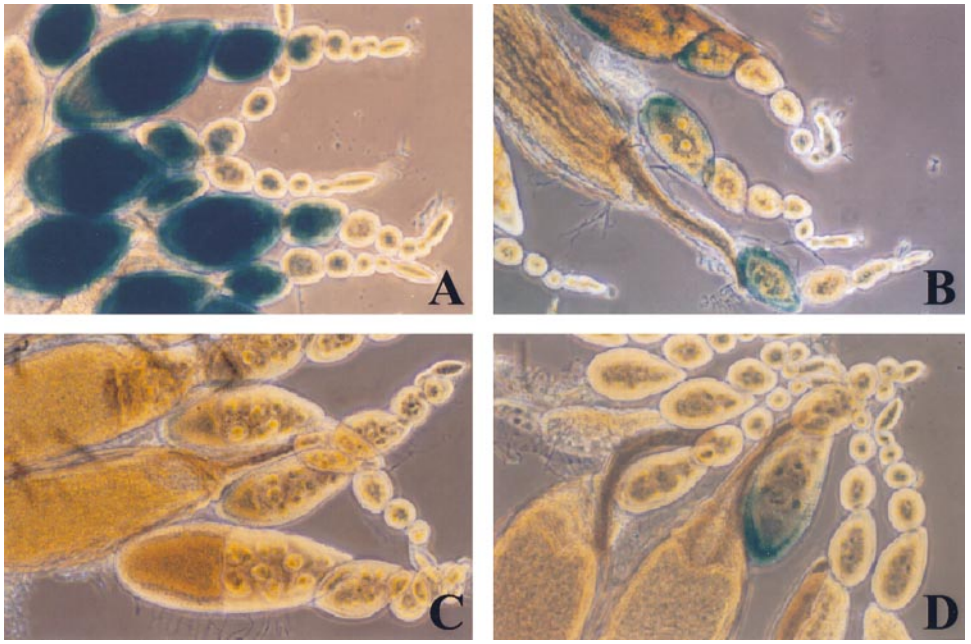

Figure 6.—Repression assay of the *P-lacZ* in *PLH3*. Each picture shows the staining of ovaries of G_1 females from the cross of tested females with *PLH3* males, reared at 20°. Tested females: (A) Canton^y; (B) *Lk-P*(1A); (C) *P-1152*; (D) *NA-P*(1A). Staining was performed as in Figure 5. The *PLH3* reporter construct is driven by the *hsp70* promoter (see Figure 4) and expression was induced by a 1-hr-30-min heat shock at 31° followed by 1 hr at 25° performed on adults. Without heat shock, the progeny of all crosses failed to show any staining (data not shown). The cytoplasmic staining observed with *PLH3* might result from the increased size of the fusion protein or from a conformational change that affects nuclear localization.

TABLE 2

Analysis of GD repression

	Maternal inheritance		Sensitivity to *Su(var)205*		Thermosensitivity aging at		
Tested lines	P-MI	M-MI	(*Cy*/+)	(*Su(var)*/+)	nt	18°	29°
Lk-P(1A)	2.2	94.3	8.7	97.0	2.2	42.9	1.2
	2.9	4.3	6.7	2.3	2.9	9.4	2.1
	(9)	(9)	(10)	(10)	(9)	(8)	(5)
NA-P(1A)	74.4	99.8	70.0	95.2	58.3	81.0	3.1
	10.4	0.4	21.3	13.3	7.0	8.8	3.9
	(12)	(12)	(14)	(13)	(4)	(6)	(6)

All results are GD sterility percentages. The mean percentage of GD sterility (first line), the standard deviation over replicates (second line), and the number of replicates performed (in parentheses, third line) are given. For each replicate test cross, 50–100 ovaries were examined. For the maternal inheritance assay, the A* test cross was performed on sets of 5–10 G_1 females from the G_0 cross (tested females × Canton^y males, denoted P-MI, or Canton^y females × tested males, denoted M-MI) performed at 20°. For the *Su(var)205* sensitivity assay, the G_0 cross was tested females × *Su(var) 205*^{4}/*Cy* males at 20°. The regulatory properties of the two kinds of G_1 females (*Cy*/+ and *Su(var)*/+, respectively) were tested with the A* assay. For the thermosensitivity assay, the G_0 cross was tested females × Canton^y males at room temperature (~24°). The A* cross was performed on sets of 5–10 G_1 females subjected to different aging treatments before mating. nt, no treatment (flies tested at emergence); 18°, 15 days of aging at 18°; 29°, 10 days of aging at 29°. Results obtained with *Lk-P*(1A) are from Ronsseray *et al.* (1991, 1996).

nearly complete repression ability (3.1%) after 10 days at 28.5°. Thus G_1 females from crosses between M and *NA-P*(1A) individuals show strong thermosensitivity in the determination of their repression ability.

Ability of *NA-P*(1A) to transmit a pre-P cytotype: Genetic experiments have previously shown that the *Lk-P*(1A) line is able to transmit a maternally inherited component, not linked to the presence of any *P* element, called pre-P cytotype (Ronsseray *et al.* 1993). We tested the ability of *NA-P*(1A) to produce such a component (Figure 7). The two reciprocal crosses between individuals from *NA-P*(1A) and an M line [*Muller-5* (*M5*)] were performed at 20°. The *M5* line has a balancer of the *X* chromosome marked with a semidominant mutation (*Bar*). The G_0 cross with *NA-P*(1A) females is referred to as the "P-grandmother" (P-GM) cross and the reciprocal cross is referred to as the "M-grandmother" (M-GM) cross. G_1 females from the P-GM cross are expected to have significant repression ability due to maternal inheritance (see Table 2, column 1) but G_1 females from the M-GM cross are expected to have weak repression ability. This was confirmed by the A* assay performed on G_1 females (data not shown). We have tested whether the *M5* gamete produced by P-GM G_1 females can transmit a "memory" of this repression ability although this gamete does not carry any *P* element. G_1 females were crossed to *Lk-P*(1A) males and the repression ability of G_2 females that inherited the *M5* chromosome was tested with the A* assay. Such a memory is expected to stimulate the repression ability of the paternally introduced *Lk-P*(1A) chromosome. As a control, the *M5*-bearing gamete from M-GM G_1 females was similarly analyzed. Figure 7 shows that G_2 females that inherit the *M5* chromosome from P-GM G_1 females have significantly stronger repression ability than corresponding females produced by M-GM G_1 females. The difference is highly significant as determined by the Mann-Whitney test ($P < 0.01$). The two *M5*-bearing oocytes (although devoid of *P* elements in both cases) do not have the same capacity to stimulate *P* repression when regulatory *P* elements are introduced by the spermatozoid. This illustrates the capacity of the P-GM G_1 females to transmit the pre-P cytotype. The same experiment with *Lk-P*(1A) in G_0 [instead of *NA-P*(1A)] produced a weaker percentage of GD sterility (P-GM = 4.2%, $s = 3.3$, $n = 13$; M-GM = 40.4%, $s = 19.1$, $n = 15$; Ronsseray *et al.* 1993). These results therefore show that *NA-P*(1A) is able to elicit the strictly maternally transmitted component of *P* repression termed the pre-P cytotype.

The foregoing genetic analysis shows that the repression ability of *NA-P*(1A) resembles that of *Lk-P*(1A) but is weaker. Furthermore, the *NA-P*(1A) and *Lk-P*(1A) strains differ in their effects on *P-lacZ* repression in the germline.

DISCUSSION

***NA-P*(1A), a new *P*-element structure at the site 1A, exerts unusual *P*-repression capacities:** The naturally occurring *P* element *NA-P*(1A) located at 1A, isolated from a Tunisian population, is a nonautonomous 5′-truncated *P*-element variant. Like the autonomous regulatory *P* elements at 1A previously isolated from French and Russian populations, *NA-P*(1A) (i) strongly represses dysgenic sterility, (ii) is capable of eliciting the pre-P cytotype, and (iii) has a repression ability apparently restricted to the germline. Furthermore, *NA-P*(1A) strongly represses *P*-element excision and its ability to repress dysgenic sterility is maternally transmitted.

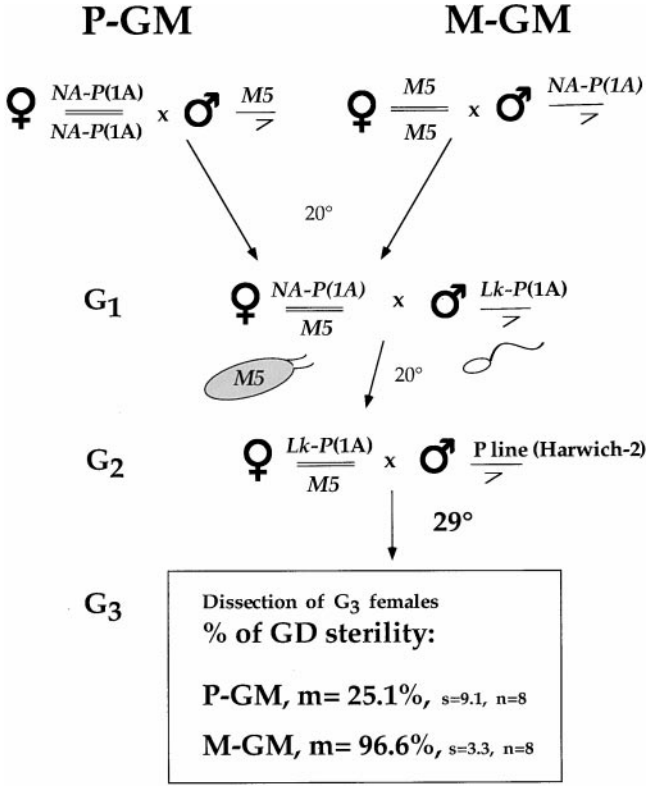

Figure 7.—Pre-P cytotype assay. *M5*, the *Muller-5* balancer chromosome from an M strain; P-GM, P grandmother; M-GM, M grandmother. The regulatory properties of the G_2 females were tested by crossing sets of these females with P strain males (Harwich-2) at 29°; *m*, *s*, and *n* are the mean percentage of GD sterility, the standard deviation over replicates, and the number of replicates performed, respectively.

However, repression by *NA-P*(1A) is impaired by a mutant allele of *Su(var)205*. Unlike the autonomous regulatory *P* elements studied previously, the ability of *NA-P*(1A) to repress a *P-lacZ* transgene depends on the structure of the transgene. *NA-P*(1A) acts as a strong repressor of *lacZ* reporter expression in the *PLH3* strain. As shown in Figure 4, this construct consists of a large *P* sequence upstream of the *lacZ* fusion. Conversely, *NA-P*(1A) does not repress a typical *P-lacZ* construct in line *BQ16*, composed only of a 5′ section of the canonical *P* element absent from the *NA-P*(1A) element. Apparently, the *NA-P*(1A) repression capacity appears to depend on homology between itself and the euchromatic *P-lacZ* target.

Is the *P*-element repression ability observed in the *NA-P*(1A) line caused by the expression of a putative *HeT-A-P* fusion protein? The *P* element of *NA-P*(1A) has not been completely sequenced but restriction enzyme mapping allowed us to localize sites at the positions corresponding to the canonical *P* element for all enzymatic sites tested downstream of the *Ava*II site at nt 1045 (Figure 1). This suggests that the remaining *P* sequence in *NA-P*(1A) is similar to the corresponding sequence in the canonical *P* element. In spite of the fact that the telomeric *NA-P*(1A) *P* element is devoid of sequences homologous to the canonical *P* promoter, this element might be transcriptionally active. *NA-P*(1A) expression could be driven from an external flanking promoter in the adjacent *HeT-A* element (Figure 2). Using promoter mapping studies, Danilevskaya *et al.* (1997) have shown that the 3′ section of a *HeT-A* sequence acts as a promoter when fused to a *lacZ* reporter gene. In addition, primer extension analysis has indicated that the transcripts extended to a position 62 nt upstream of the 3′ end of the *HeT-A* element. A homologous sequence is found in the *HeT-A* segment located just upstream of the *NA-P*(1A) *P* element. A potential ATG start codon for translational initiation is also found between this position (62 nt upstream) and the *P* sequence, which can give rise to an in-frame fusion open reading frame (ORF). This ORF has 18 amino acids (aa) belonging to the *HeT-A* sequence fused with half of *P*-element exon 1. It is followed by exon 2 of *P* (and exon 3 if the last *P* intron is removed). *NA-P*(1A) could have the coding capacity for a 43-kD (or a 64-kD) protein, similar to an N-terminal truncated *P* polypeptide devoid of the first 221 aa encoded by exon 0 and part of exon 1. Thus, the deduced *P* protein of *NA-P*(1A) lacks the DNA-binding domain of the *P* polypeptides that resides in the 88 N-terminal amino acids (Lee *et al.* 1996; Lee and Rio 1998). This amino acid segment includes a CCHC putative metal-binding motif that is required for site-specific DNA binding (Miller *et al.* 1995; Lee and Rio 1998). The *NA-P*(1A) deduced protein also lacks the coiled-coil domain within the first leucine zipper (Andrews and Gloor 1995; Miller *et al.* 1995; Belenkaya *et al.* 1998). Hence it seems reasonable that the putative polypeptide of the *NA-P*(1A) element would not be able to recognize and block a canonical *P* promoter. However, the putative *NA-P*(1A) protein still retains two additional leucine zippers located in exons 1 and 2 of the canonical *P* (Rio 1990). In addition, the fusion of *HeT-A* with *P* results in the formation of a presumptive new leucine zipper (data not shown). Following this line of argument, the deduced *NA-P*(1A) protein might still be capable of repressing *P*-element mobility via protein-protein interaction, *e.g.*, by heterodimer formation with the P transposase (Rio 1990).

The ability of *NA-P*(1A) to elicit the pre-P cytotype suggests that a diffusible product plays a role in its repression capacity. It was first postulated that the pre-P cytotype in *Lk-P*(1A) corresponds to a deposit of a polypeptide repressor in the mature oocyte (Ronsseray *et al.* 1993). The *P* elements of *Lk-P*(1A) are expressed since transposase activity is genetically detectable using the sn^w excision assay (Ronsseray *et al.* 1991, 1996). However, the *Lk-P*(1A) expression is undetectable by Western analysis using an antibody against P proteins (S. E. Roche, S. Misra and D. C. Rio, personal communication). This suggests that such a low level of protein cannot be detected easily by immunological analyses

and thus we cannot exclude that *NA-P*(1A) females produce and deposit a *P*-encoded polypeptide in oocytes.

Does a *trans*-silencing effect play a role in the *P*-repression ability of *NA-P*(1A)? A case of homology-dependent transgene silencing, induced by telomeric transgenes, has been previously reported in tobacco (Vaucheret 1993, 1994). In this study, a plant numbered "*271*" carried a telomeric silencer locus that has multiple copies of a chimeric transgene: an antisense *nitrite reductase* cDNA (*RiN*) under the control of the *35S* promoter of the cauliflower mosaic virus. This telomeric silencing locus is highly methylated and is able to silence any euchromatic gene under the control of the same promoter (Vaucheret 1993, 1994). The authors hypothesized that the silencing capacity of the telomeric *271* locus is due to the location of this locus. The telomeric position could allow a rapid scanning of the genome; the silencer locus could then recognize a homologous target and inactivate it (Matzke *et al.* 1994). This transcriptional silencing is correlated to the methylation of the target transgene (Vaucheret 1993). As the genome of *D. melanogaster* was not found to be methylated at a detectable level, the structural modifications of chromatin involved in such a model for a *trans*-silencing effect in Drosophila could be mediated by *trans*-heterochromatinization (Roche and Rio 1998).

Alternatively, transgene silencing in tobacco can also involve a post-transcriptional repression component (Park *et al.* 1996; Vaucheret *et al.* 1996). It is proposed that the telomeric transgene produces RNAs that lead to the degradation of the homologous RNAs. In Drosophila, it is also possible that the pre-P cytotype is caused by a similar RNA-mediated mechanism. *P*-RNAs produced by the telomeric *P* insertion could be aberrant RNA or double-stranded RNA (dsRNA) molecules, which could induce cosuppression. In fact, post-transcriptional RNA-mediated cosuppression has been recently shown to regulate transposable element activity in Drosophila. As shown by Jensen *et al.* (1999a,b), noncoding RNAs of the *D. melanogaster* retrotransposon *I* can efficiently repress *I*-element activity *in vivo.* This effect is inducible by introducing modified *I* elements, which produce either noncoding or antisense RNAs. In addition, Kennerdell and Carthew (1998) have shown that dsRNA in Drosophila is capable of causing a null phenotype of the *frizzled* gene. Regarding *P* repression, Simmons *et al.* (1996) have shown that expression of antisense RNAs can lead to partial *P* repression. Reverse transcriptase (RT)-PCRs in *Lk-P*(1A) adults allowed detection of *P* transcripts (Roche *et al.* 1995). In our survey, we were unable to detect *P*-element transcription via Northern analysis for both *Lk-P*(1A) and *NA-P*(1A). Hence, we cannot exclude the possibility that the *P* element of *NA-P*(1A) is transcribed at a very low level in some tissues or developmental stages and the sensitivity of the method used in our study is insufficient for detecting such a weak expression level.

Under these two nonmutually exclusive models, *i.e.*, transcriptional and post-transcriptional silencing, target sequence homology seems to play a crucial role. The *NA-P*(1A) transgene repression capacities, which depend on homology with the target transgene, evoke such *trans*-silencing phenomena. Further experiments will be necessary to determine the nature of the molecular support implied here.

The molecular genesis of the telomeric *NA-P*(1A) *P*-element insertion: At its 3′ end, the *NA-P*(1A) element is flanked by a *TAS* element, a structural feature resembling the insertion sites of the *P* elements of *Lk-P*(1A) and of *Ch-P*(1A) (Ronsseray *et al.* 1996). In general, *P* elements are known to insert preferentially inside *TAS* elements, thus providing a hotspot for *P* insertions (Karpen and Spradling 1992). The molecular position of *NA-P*(1A) within a *TAS* repeat differs from those of *Lk-P*(1A) and *Ch-P*(1A) (Ronsseray *et al.* 1996). This shows that the different naturally occurring *P*(1A) insertions sampled in natural populations are not identical by descent, but come from independent transpositional events. In the *NA-P*(1A) line, the *P* element lacks the first 871 bp. The *HeT-A-P* fusion observed here can therefore be explained by the following scenario: a full-sized *P* element is first inserted inside *TAS*, then a terminal deletion occurs, removing the upstream *TAS* section and the 5′ end of the *P* element (5′ inverted repeat, exon 0 and half of exon 1); an incomplete replication of the chromosome over generations may have helped to achieve this. Finally, a *HeT-A* copy transposes at the telomere, thus healing the broken *X* chromosome. *In situ* hybridization on polytene chromosomes with a *TAS* probe showed signals at 1A in all three *P*(1A) lines tested [*i.e.*, *Lk-P*(1A), *Ch-P*(1A), and *NA-P*(1A)], but the signal intensity obtained on the tip of the *X* chromosome for *NA-P*(1A) was significantly weaker compared to those of *Lk-P*(1A) and *Ch-P*(1A) (data not shown), suggesting that the 1A *TAS* cluster of *NA-P*(1A) is shorter than those of the two other *P*(1A) lines. This observation is consistent with our terminal deletion model. Furthermore, the genomic organization of the telomeric *NA-P*(1A) element, derived from a natural sample, resembles that of some telomeric *P*-reporter insertions that originated in the laboratory. The *P-w*^{var} line harbors a *P-w*$^{+}$ transgene in one of the telomeres of the second chromosome (2L). This transgene was found to be truncated at its 5′ end. Like *NA-P*(1A), it is flanked in 3′ by a *TAS* and in 5′ by a *HeT-A* sequence (H. Biessmann and J. Mason, personal communication). Furthermore, in the *P-833* line harboring a *P-w*$^{+}$ transgene in the telomere of the third chromosome (3R), Sheen and Levis (1994) showed that spontaneous terminal deletions and incomplete replication removed a part of the transgene and the flanking distal DNA; the telomere was then rescued by a terminal *TART* insertion.

From an evolutionary point of view, the *NA-P*(1A) insertion is an immobile regulatory *P* element since it

lacks the 5′ terminal repeat: such a copy could confer a selective advantage by suppressing the deleterious effects caused by *P*-induced hybrid dysgenesis. Furthermore, a molecular structure like the one described in the present study could thus serve as a starting point for an evolutionary process termed "molecular domestication" (Miller *et al.* 1992): independent molecular transitions of formerly active *P* elements into chromosomal neogenes have indeed been described earlier in other Drosophila species (Paricio *et al.* 1991; Miller *et al.* 1992, 1995; Nouaud and Anxolabéhère 1997; Nouaud *et al.* 1999; for recent review, see Miller *et al.* 2000).

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